Recombinant VP4 of Human Rhinovirus Induces Permeability in Model Membranes[▽]

Matthew P. Davis,^{1†} Graham Bottley,¹ Lucy P. Beales,² Richard A. Killington,¹
David J. Rowlands,¹ and Tobias J. Tuthill^{1*}

Institute for Molecular and Cellular Biology, Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, United Kingdom,¹ and iQur Ltd., Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, United Kingdom²

Received 17 May 2007/Accepted 21 January 2008

In common with all nonenveloped viruses, the mechanism of picornavirus membrane penetration during cell entry is poorly understood. The small, myristylated capsid protein VP4 has been implicated in this process. Here we show that recombinant VP4 of human rhinovirus 16 has the ability to associate with and induce membrane permeability in otherwise intact liposomes. This provides further evidence that VP4 plays a key role in picornavirus cell entry.

Human rhinoviruses (HRVs) are a major cause of the common cold and belong to the picornavirus family. Picornaviruses provide good models for studying nonenveloped virus cell entry since they are among the simplest viruses of this type, they have been well characterized biochemically, and the structures of many, including several serotypes of HRV, have been solved at high resolution (20, 23, 27, 33, 34). Picornaviruses have icosahedral capsids composed of 60 copies of each of the structural proteins, VP1, VP2, VP3, and the small N-terminally myristylated protein VP4, which together enclose a single-stranded positive-sense RNA genome of between 7 and 8.5 kb. Cell entry by HRV involves uptake by the clathrin-mediated endocytic pathway (10, 29), followed by uncoating and delivery of the genome across the endosome membrane into the cytoplasm.

There are over 100 serotypes of HRV, which can be grouped according to receptor usage (32). Minor-group viruses such as HRV2 use the low-density lipoprotein receptor family as receptors. They are acid labile and have an absolute dependence on low pH for uncoating (24). During infection, the process of uncoating leads to the delivery of the HRV2 genome into the cytoplasm from within the intact endosome, through a size-selective pore in the endosome membrane (3, 25). However, the mechanism of the formation of this pore remains unknown.

Major-group viruses such as HRV14 and HRV16 use intercellular adhesion molecule 1 (ICAM-1) as their receptor. They are also generally acid labile but, unlike the minor-group viruses, do not have an absolute dependence on low pH for uncoating (1). For example, receptor binding at neutral pH is sufficient to initiate the uncoating of HRV14 *in vitro* (19), similar to the situation with the closely related poliovirus (PV). During infection, uncoating of HRV14 leads to the delivery of

the genome to the cytoplasm by a mechanism that appears to completely disrupt the endosome membrane (28). The mechanism for producing such a membrane alteration remains unclear.

In addition to these findings with HRV, studies with PV have also provided information about early events in virus entry (4, 6, 18). During the uncoating of either HRV or PV, native virus (which sediments at ~160S) is converted to an altered particle (~135S). For PV, VP4 has been released from the altered particle to become associated with the membrane (8, 31). The hydrophobic N termini of the VP1 proteins have also become externalized and allow the particle to interact directly with the membrane (12, 31). Further alterations in the particle ultimately result in an empty capsid (~80S) which in the case of HRV has lost some or all of its VP4 and contains pores at the fivefold axes through which the RNA genome is thought to have been released (17). The mechanisms of the release of RNA from the particle, the permeabilization of the endosome membrane, and the coordination of these events (to provide safe passage of RNA from the particle to the cytoplasm) remain unclear.

The interaction of VP4 with membranes following its release from the particle suggests a potential role for VP4 in the membrane alterations required for genome transfer. Indeed, a specific mutation in VP4 of PV prevents the formation of membrane-spanning channels in model membranes and also prevents productive genome delivery to the cytoplasm of the cell (8). There is therefore evidence for a link between VP4-mediated membrane permeability and genome delivery. However, the precise role of VP4 in picornavirus genome delivery has yet to be established.

We describe here the expression of recombinant HRV16 VP4 as a glutathione *S*-transferase (GST) fusion protein, VP4GST, and an investigation of its ability to interact with and induce permeability in lipid membranes in the form of liposomes.

Preparation of recombinant VP4. The sequence encoding HRV16 VP4 was amplified from an infectious clone of the virus (21) by PCR using primers VP4F (5'ATATGCTAGCC TCGCCATGGCGCTCAAGTATCTAGACAGAATGTTG

* Corresponding author. Mailing address: Institute for Molecular and Cellular Biology, Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, United Kingdom. Phone: 44 113 3435643. Fax: 44 113 3435638. E-mail: t.tuthill@leeds.ac.uk.

† Present address: Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, United Kingdom.

[▽] Published ahead of print on 6 February 2008.

FIG. 1. (A) Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified unmyristylated VP4GST (lane 1), myristylated VP4GST (lane 2), and GST (lane 3). The positions of molecular mass markers (kDa) are indicated. The image was captured with a gel documentation system (Syngene). (B) VP4GST associates with liposomes. Lanes 1 to 10 represent fractions, from top to bottom, of Ficoll gradients, immunoblotted with GST-reactive serum. Liposomes banded at fraction 2. The positions of molecular mass markers (kDa) are indicated. Immunoblots were exposed to film and scanned (Epson 4870).

G3') and VP4R (5'GGATCCACGCGGAACCAGATGGTG ATGGTGATGGTGTTGCAGAGTGGGTATGCCTTTCTC C3'). GST was amplified by PCR using primers GSTF (5'CA CCATCACCATCACCATCTGGTTCGCGTGGATCCAT GTCCCCTATACTAGGTTATTGG3') and GSTR (5'TATA GCGGCCGCCTCGAGTCAATCCGATTTTGGAGGATG G3') from pGEX 4T-1 (Invitrogen). These PCR products were used as overlapping templates (underlined regions denote complementary primer sequences) in a further PCR to produce a cDNA encoding VP4GST, which was cloned into pET-23d (Novagen) using restriction sites NcoI and XhoI, producing pET-VP4GST. Unmyristylated VP4GST or GST was expressed in BL21(DE3) Rosetta *Escherichia coli* transformed with pET-VP4GST or pGEX 4T-1, respectively. Myristylated VP4GST was produced in the same host by cotransformation with a plasmid encoding *N*-myristyltransferase (pET-NMT) (9, 11). Expression was induced by the addition of 1 mM isopropyl β -D-1-thiogalactopyranoside. Myristic acid was added to a final concentration of 5 μ g/ml to cells cotransformed with pET-NMT. Soluble VP4GST or GST was purified from lysates by glutathione affinity chromatography using standard methods and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1A). The myristylation of VP4GST was confirmed using slow-crystallization matrix-assisted laser desorption ionization mass spectrometry (2). By this method, the mass of myristylated VP4GST was measured as $34,670 \pm 29$ Da and that of unmyristylated VP4GST as $34,472 \pm 57$ Da. The difference in molecular mass is consistent with the addition of myristic acid (205 Da).

VP4GST-membrane interaction is mediated by VP4. Following its externalization from PV, VP4 has been shown to partition into either cellular (8) or liposome (31) membranes. We therefore determined whether the recombinant VP4GST used in this study could also interact with liposomes. Liposomes were formed from phosphatidic acid (PA) and 0.5% (wt/vol) lissamine rhodamine B-labeled phosphatidylethanolamine by

previously described protocols (30, 31). Briefly, chloroform was evaporated to leave a thin film of lipid that was dried under vacuum before being rehydrated in 100 mM KCl–10 mM HEPES, pH 7.4. Unilamellar liposomes were formed by extrusion through a 400-nm-pore-size filter. To detect protein-membrane interactions, liposomes (100 μ g, approximately 1.4 mM) were mixed with VP4GST or GST at approximately 3 μ M and incubated at room temperature for 30 min before membrane-associated protein was separated from free protein by lipid flotation through a Ficoll gradient, as previously described (31). Gradients were fractionated from the top and analyzed for liposomes by rhodamine fluorescence (544/590 nm) and for protein by Western blotting using GST-reactive antiserum (15). The majority of either myristylated or unmyristylated VP4GST comigrated with liposomes (Fig. 1B) to a Ficoll/buffer interface (corresponding to gradient fraction 2) and was therefore deemed to be liposome associated. GST showed no such lipid association in this assay, confirming that the association of VP4GST with liposomes was mediated by the VP4 component. Some protein preparations contained a low-molecular-weight form of VP4GST (Fig. 1A) that was reactive with both anti-VP4 and anti-GST sera (data not shown) and that also retained the ability to interact with liposomes (Fig. 1B). Higher-molecular-weight material was also detected in liposome fractions (Fig. 1B) and may represent multimeric forms of VP4GST.

VP4GST induces membrane permeability in a concentration- and myristylation-dependent manner. There is existing evidence for the involvement of VP4 in the formation of membrane channels by PV (8). Having shown that VP4GST associated with liposomes, we therefore examined the effect of this interaction on membrane permeability by using a liposome dye release assay. Liposomes comprising a 1:1 molar ratio of PA and phosphatidylcholine with 0.5% (wt/vol) rhodamine B-labeled phosphatidylethanolamine and enclosing 50 mM (self-quenching) carboxyfluorescein (CF) were generated by rehy-

dration in 50 mM CF–10 mM HEPES, pH 7.4 (PA liposomes were refractory to preparation with high concentrations of internal CF). After extrusion, CF-containing unilamellar liposomes were isolated from extraneous dye by washing four times in 107 mM NaCl–10 mM HEPES (pH 7.3) by pelleting at $100,000 \times g$ and 20°C for 15 min. The level of rhodamine fluorescence in purified liposome preparations was used to estimate the lipid concentration relative to the initial rehydrated lipid.

Liposomes (25 μ M lipid) were mixed at approximately 25°C with VP4GST or GST or mock treated. Membrane permeability was detected as the movement of quenched CF from inside the liposome to become dequenched on release to the outside, with a subsequent increase in fluorescent signal (485/520 nm) that was recorded using a 96-well fluorimeter (BMG FluoStar). At a concentration of 300 nM, both myristylated and unmyristylated forms of VP4GST induced the relatively rapid release of CF from liposomes (Fig. 2A), with the initial rates of release being approximately six- to sevenfold higher than the background level of release induced by GST (Fig. 2C). At this concentration, the presence of myristate therefore appeared not to be required for VP4 function in this assay. When used at a 10-fold-lower concentration (30 nM), myristylated VP4GST still induced a more rapid and extensive permeability than GST; however, unmyristylated VP4GST was no more effective than GST alone (Fig. 2B and C). Thus, at the lower concentration, myristylation of VP4 is required for optimal activity. When the concentration was further reduced to 3 nM, CF release could not be reproducibly detected. Similar overall results were obtained at 37°C (data not shown), suggesting that physiological temperature is not a requirement for membrane permeability in this assay. The kinetics of VP4-induced CF release appeared broadly comparable to that of CF release induced by the well-characterized pore-forming peptide melittin (Fig. 2B) (26).

Liposomes permeabilized by VP4GST remain intact. Previous studies have reported either the formation of size-selective membrane pores in intact endosomes during entry of HRV2 (3, 25) or the disruption of endosomes during entry of HRV14 (28). In order to determine whether liposomes remained intact or were disrupted by interaction with VP4GST, we investigated their properties by using flow cytometry.

Liposomes (25 μ M lipid) containing 1.6 mM (unquenched) CF were mixed at room temperature with VP4GST or GST or were mock treated. After 10 min, anti-GST antibodies (murine; Serotec) were added, followed after an additional 10 min by labeled secondary antibodies (anti-mouse Alexafluor 633; Invitrogen). After a further 10 min, liposomes were analyzed by flow cytometry (BD FACSaria and FACSDiva). Liposomes were detected by forward and side scattering of light and by the fluorescent signal derived from enclosed CF (488/525 nm). The interaction between proteins and liposomes was detected by the additional fluorescent signal from immunostaining with anti-GST (633/647 nm).

After the addition of VP4GST, liposomes were simultaneously found to have bound high levels of protein, increased in granularity, and lost the majority of their internal contents while remaining intact (Fig. 3). They could be physically sorted into distinct populations by, for example, their reduced internal fluorescence, and liposomes that had been permeabilized

FIG. 2. VP4GST-induced membrane permeability measured by the release and fluorescent dequenching of CF from liposomes. (A) Time course of release induced by 300 nM myristylated (black circles) or unmyristylated (gray circles) VP4GST and by GST alone (open circles). The release induced by Triton X-100 (triangles) served as a control for the complete disruption of liposomes. The data shown are representative of multiple experiments. (B) Time course of release induced by 30 nM myristylated (black circles) or unmyristylated (gray circles) VP4GST and by GST alone (open circles). The release induced by 100 nM melittin (crosses) is shown for comparison. The data shown are representative of multiple experiments. (C) Relative initial rates of release for myristylated VP4GST (myr), unmyristylated VP4GST (un), and GST alone, as calculated by fitting a straight line to the initial four data points of the time course. Error bars indicate standard deviations ($n = 3$).

could still be sorted and reanalyzed, therefore confirming liposome integrity (data not shown). The formation of distinct populations of liposomes with either low or high levels of protein binding indicated that there may be a cooperative interaction of proteins with the membrane. The release of internal contents from within intact liposomes strongly suggests a controlled mechanism of permeability such as the formation of a membrane pore. At protein concentrations of 30 nM, typically 40% of intact liposomes had lost on average 90% of their internal fluorescence, compared with 10 to 15% of liposomes mixed with GST alone. The loss of the majority of

FIG. 3. Liposomes permeabilized by VP4GST remain intact by flow cytometry. Analyses of liposomes alone (A) and after the addition of 30 nM myristylated VP4GST (B), unmyristylated VP4GST (C), or GST (D) are shown. Dot plots in the left column show detection of liposomes by forward and side light scattering; after addition of protein (B, C, and D), a proportion of liposomes (purple) have increased granularity (side scatter) relative to liposomes alone (A). Dot plots in the middle column show two populations of liposomes: low-level signal for protein binding with high-level CF internal fluorescence (orange) and high-level protein binding with decreased CF internal fluorescence (purple) as a result of induced permeability and release of CF. This analysis was not carried out with liposomes alone (A). The histograms in the right column show the distribution of CF fluorescence for the populations (orange and purple) described in the dot plots. The data shown are representative of three independent experiments.

CF from only a proportion of liposomes suggested that permeability was an all-or-nothing event, perhaps requiring a critical number of molecules per liposome. In order to calculate the VP4GST/liposome ratio, we first estimated the liposome

concentration by calculating the number of lipid molecules per liposome based on the relative surface areas of liposomes ($500 \times 10^3 \text{ nm}^2$) and lipid head groups (0.7 nm^2). At a protein concentration of 30 nM, the VP4GST/liposome ratio was ap-

proximately 2,000:1. However, the number of functional pore-forming units per liposome would be only a fraction of this, because for pore formation to occur, a number of molecules would be required to specifically interact as a multimeric complex.

In contrast to following the kinetics of dye release in real time (Fig. 2), differences between the effects of myristylated and unmyristylated forms of VP4GST were not discernible by flow cytometry (Fig. 3), although both proteins clearly induced permeability beyond that of GST alone. The inability of this assay to discriminate between the presence or absence of myristate presumably reflects the fundamental differences between the endpoint analysis of the flow cytometry and the kinetic analysis of the real-time assay.

At protein concentrations 10-fold higher (300 nM), two very similar populations of liposomes were also observed by flow cytometry (data not shown). However, at this increased concentration of protein, no significant additional effect was observed over that already seen at 30 nM. This was in contrast to the findings of the kinetic assay, where the higher protein concentration produced a more rapid and extensive permeability. It is possible that the increased rate of CF release seen at 300 nM in the kinetic assay was due to a large-scale membrane disruption, such that these liposomes were no longer intact and were below the size limit for detection by flow cytometry. In support of this hypothesis, we have observed the release of high-molecular-weight dextran molecules from liposomes together with electron microscopy evidence of their disruption in the presence of very high concentrations of VP4GST (data not shown).

It is tempting to speculate that permeability induced by low concentrations of VP4GST is due to the formation of discrete membrane pores, which may require myristylated VP4, while permeability at higher concentrations may be due to a less specific membrane disruption which is independent of myristylation. This hypothesis would also reconcile the apparently conflicting evidence for pore formation by HRV2 (and PV) and endosome disruption by HRV14 during the entry process. Existing analyses of several picornavirus empty particles (13, 14, 16–19) suggest that variable proportions of the 60 VP4 molecules present in virions are released during entry. This could influence the endosomal VP4 concentration, creating a preference for either pore formation or membrane disruption. Sixty VP4 molecules in a 350-nm-diameter endosome would generate an estimated concentration of 14 μ M (or less if release of VP4 was incomplete), which is reasonably close to the concentrations of recombinant protein used in this study.

Alternatively, these different modes of membrane permeability may simply be the result of variation between different viruses. HRV2 and HRV16 are closely related and differ by only 5 amino acids in VP4; HRV2 and HRV14 are more distantly related and differ by 31 amino acids. However, the properties of variant amino acids are largely conserved, with the result that structure predictions for these proteins are indistinguishable. Despite this, there may be subtle functional differences between these VP4 proteins. The effects of sequence variation in VP4, including the effect of VP4 mutations known to influence the properties of PV VP4, are the subject of further investigation.

The results of this study suggest that VP4 has the intrinsic

ability to bind lipid membranes, resulting in their permeabilization. When the N-terminal myristylation of VP4 was first demonstrated, it was suggested that this modification may be important for membrane association (7). More recently the myristylation of a recombinant VP4-green fluorescent protein fusion protein was shown to be required for membrane targeting within the cell (22). Our current findings provide the first confirmation of a specific functional role for the myristate in VP4 membrane activity. This report provides further evidence that VP4 is directly involved in mediating membrane penetration during cell entry, in agreement with previous hypotheses (5, 18). The possibility that VP4-liposome interactions may result in alternate, concentration-dependent mechanisms of membrane permeability provides a potential explanation for the differences observed between the entries of otherwise very similar viruses.

We thank Roger Clegg for providing pET-NMT, Dean Clarke and Corine StGelais for GST-reactive serum, and the University of St. Andrews Mass Spectrometry Facility.

This work was funded by the Wellcome Trust and the Medical Research Council.

REFERENCES

1. Bayer, N., E. Prchla, M. Schwab, D. Blaas, and R. Fuchs. 1999. Human rhinovirus HRV14 uncoats from early endosomes in the presence of bafilomycin. *FEBS Lett.* **463**:175–178.
2. Botting, C. H. 2000. The use of the slow crystallisation method to improve matrix-assisted laser desorption/ionisation time-of-flight signals for larger proteins. *Rapid Commun. Mass Spectrom.* **14**:2030–2033.
3. Brabec, M., D. Schober, E. Wagner, N. Bayer, R. F. Murphy, D. Blaas, and R. Fuchs. 2005. Opening of size-selective pores in endosomes during human rhinovirus serotype 2 in vivo uncoating monitored by single-organelle flow analysis. *J. Virol.* **79**:1008–1016.
4. Brandenburg, B., L. Y. Lee, M. Lakadamyali, M. J. Rust, X. Zhuang, and J. M. Hogle. 2007. Imaging poliovirus entry in live cells. *PLoS Biol.* **5**:e183.
5. Bubeck, D., D. J. Filman, N. Cheng, A. C. Steven, J. M. Hogle, and D. M. Belnap. 2005. The structure of the poliovirus 135S cell entry intermediate at 10-angstrom resolution reveals the location of an externalized polypeptide that binds to membranes. *J. Virol.* **79**:7745–7755.
6. Bubeck, D., D. J. Filman, and J. M. Hogle. 2005. Cryo-electron microscopy reconstruction of a poliovirus-receptor-membrane complex. *Nat. Struct. Mol. Biol.* **12**:615–618.
7. Chow, M., J. F. Newman, D. Filman, J. M. Hogle, D. J. Rowlands, and F. Brown. 1987. Myristylation of picornavirus capsid protein VP4 and its structural significance. *Nature* **327**:482–486.
8. Danthi, P., M. Tosteson, Q. H. Li, and M. Chow. 2003. Genome delivery and ion channel properties are altered in VP4 mutants of poliovirus. *J. Virol.* **77**:5266–5274.
9. Dennis, C. A., A. Baron, J. G. Grossmann, S. Mazaleyra, M. Harris, and J. Jaeger. 2005. Co-translational myristylation alters the quaternary structure of HIV-1 Nef in solution. *Proteins* **60**:658–669.
10. DeTulleo, L., and T. Kirchhausen. 1998. The clathrin endocytic pathway in viral infection. *EMBO J.* **17**:4585–4593.
11. Duronio, R. J., E. Jackson-Machelski, R. O. Heuckeroth, P. O. Olins, C. S. Devine, W. Yonemoto, L. W. Slice, S. S. Taylor, and J. I. Gordon. 1990. Protein N-myristylation in *Escherichia coli*: reconstitution of a eukaryotic protein modification in bacteria. *Proc. Natl. Acad. Sci. USA* **87**:1506–1510.
12. Fricks, C. E., and J. M. Hogle. 1990. Cell-induced conformational change in poliovirus: externalization of the amino terminus of VP1 is responsible for liposome binding. *J. Virol.* **64**:1934–1945.
13. Goodfellow, I. G., D. J. Evans, A. M. Blom, D. Kerrigan, J. S. Miners, B. P. Morgan, and O. B. Spiller. 2005. Inhibition of coxsackie B virus infection by soluble forms of its receptors: binding affinities, altered particle formation, and competition with cellular receptors. *J. Virol.* **79**:12016–12024.
14. Greve, J. M., C. P. Forte, C. W. Marlor, A. M. Meyer, H. Hoover-Litty, D. Wunderlich, and A. McClelland. 1991. Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1. *J. Virol.* **65**:6015–6023.
15. Griffin, S. D., L. P. Beales, D. S. Clarke, O. Worsfold, S. D. Evans, J. Jaeger, M. P. Harris, and D. J. Rowlands. 2003. The p7 protein of hepatitis C virus forms an ion channel that is blocked by the antiviral drug, Amantadine. *FEBS Lett.* **535**:34–38.
16. Gromeier, M., and K. Wetz. 1990. Kinetics of poliovirus uncoating in HeLa cells in a nonacidic environment. *J. Virol.* **64**:3590–3597.

17. Hewat, E. A., and D. Blaas. 2004. Cryoelectron microscopy analysis of the structural changes associated with human rhinovirus type 14 uncoating. *J. Virol.* **78**:2935–2942.
18. Hogle, J. M. 2002. Poliovirus cell entry: common structural themes in viral cell entry pathways. *Annu. Rev. Microbiol.* **56**:677–702.
19. Hoover-Litty, H., and J. M. Greve. 1993. Formation of rhinovirus-soluble ICAM-1 complexes and conformational changes in the virion. *J. Virol.* **67**:390–397.
20. Kim, S. S., T. J. Smith, M. S. Chapman, M. C. Rossmann, D. C. Pevear, F. J. Dutko, P. J. Felock, G. D. Diana, and M. A. McKinlay. 1989. Crystal structure of human rhinovirus serotype 1A (HRV1A). *J. Mol. Biol.* **210**:91–111.
21. Lee, W. M., W. Wang, and R. R. Rueckert. 1995. Complete sequence of the RNA genome of human rhinovirus 16, a clinically useful common cold virus belonging to the ICAM-1 receptor group. *Virus Genes* **9**:177–181.
22. Martin-Belmonte, F., J. A. Lopez-Guerrero, L. Carrasco, and M. A. Alonso. 2000. The amino-terminal nine amino acid sequence of poliovirus capsid VP4 protein is sufficient to confer N-myristoylation and targeting to detergent-insoluble membranes. *Biochemistry* **39**:1083–1090.
23. Oliveira, M. A., R. Zhao, W. M. Lee, M. J. Kremer, I. Minor, R. R. Rueckert, G. D. Diana, D. C. Pevear, F. J. Dutko, M. A. McKinlay, et al. 1993. The structure of human rhinovirus 16. *Structure* **1**:51–68.
24. Prchla, E., E. Kuechler, D. Blaas, and R. Fuchs. 1994. Uncoating of human rhinovirus serotype 2 from late endosomes. *J. Virol.* **68**:3713–3723.
25. Prchla, E., C. Plank, E. Wagner, D. Blaas, and R. Fuchs. 1995. Virus-mediated release of endosomal content in vitro: different behavior of adenovirus and rhinovirus serotype 2. *J. Cell Biol.* **131**:111–123.
26. Rex, S. 1996. Pore formation induced by the peptide melittin in different lipid vesicle membranes. *Biophys. Chem.* **58**:75–85.
27. Rossmann, M. G., E. Arnold, J. W. Erickson, E. A. Frankengerger, J. P. Griffith, H. J. Hecht, J. E. Johnson, G. Kamer, M. Luo, A. G. Mosser, et al. 1985. Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature* **317**:145–153.
28. Schober, D., P. Kronenberger, E. Prchla, D. Blaas, and R. Fuchs. 1998. Major and minor receptor group human rhinoviruses penetrate from endosomes by different mechanisms. *J. Virol.* **72**:1354–1364.
29. Snyers, L., H. Zwickl, and D. Blaas. 2003. Human rhinovirus type 2 is internalized by clathrin-mediated endocytosis. *J. Virol.* **77**:5360–5369.
30. StGelais, C., T. J. Tuthill, D. S. Clarke, D. J. Rowlands, M. Harris, and S. Griffin. 2007. Inhibition of hepatitis C virus p7 membrane channels in a liposome based assay system. *Antiviral Res.* **76**:48–58.
31. Tuthill, T. J., D. Bubeck, D. J. Rowlands, and J. M. Hogle. 2006. Characterization of early steps in the poliovirus infection process: receptor-decorated liposomes induce conversion of the virus to membrane-anchored entry-intermediate particles. *J. Virol.* **80**:172–180.
32. Uncapher, C. R., C. M. DeWitt, and R. J. Colonno. 1991. The major and minor group receptor families contain all but one human rhinovirus serotype. *Virology* **180**:814–817.
33. Verdaguer, N., D. Blaas, and I. Fita. 2000. Structure of human rhinovirus serotype 2 (HRV2). *J. Mol. Biol.* **300**:1179–1194.
34. Zhao, R., D. C. Pevear, M. J. Kremer, V. L. Giranda, J. A. Kofron, R. J. Kuhn, and M. G. Rossmann. 1996. Human rhinovirus 3 at 3.0 Å resolution. *Structure* **4**:1205–1220.